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Poor Oral Health and its Neurological Consequences: Mechanisms of *Porphyromonas gingivalis* Involvement in Cognitive Dysfunction

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Abstract

Purpose of review: There is an increasing body of evidence from epidemiology and laboratory investigations on periodontal disease being a risk factor for dementia. In particular, *Porphyromonas gingivalis* infections in animal models suggest causal associations with Alzheimer's disease (AD). This review focus~~s~~es on how *P. gingivalis* infections promote the incidence of functional loss in AD.

Latest findings: The risk of the sporadic form of AD doubles when periodontitis persists for ten or more years. AD differs from other forms of dementia in that the clinical signs together with the presence of amyloid-beta (Aβ) plaques and neurofibrillary tangles must be present at autopsy. *P. gingivalis* oral infections in mice have demonstrated all of the characteristic pathological and clinical features of AD following infection ~~upon their entry~~ ~~t~~of the brain.

Summary: Multiple factors (inflammation, Aβ oligomers, and bacterial factors) are likely to disrupt neuronal communication channels (synapses) as a plausible explanation for the functional loss.

Abstract: 150 words

Bulk of article: 3,640 words

Keywords Alzheimer's disease; Periodontitis; Interaction; *P. gingivalis;* Virulence factors

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Introduction

Longstanding periodontitis, formerly known as "chronic" periodontitis has an adverse effect on a number of complex human diseases associated with longstanding inflammation [1-3]. Recent research has linked poor oral hygiene to ~~other~~ neurological conditions that manifest with dementia. Currently they include the sporadic form of Alzheimer's disease (AD), and the Lewy body Parkinson's disease (dementia) [4-6]. Amyloid-beta (Aβ) plaques are central to all forms of dementia, but are more important to AD pathology. A significant body of literature considers the Aβ plaques of AD and the α-synuclein of Lewy bodies to be antimicrobial peptides that combat infections of the brain [7-10]. This concept may provide vital clues to the occurrence of these neuropathological lesions.

Porphyromonas gingivalis

Porphyromonas gingivalis is found in the oral cavity (saliva) of all humans where it may or may not cause oral pathology, but is able to tolerate low concentrations of oxygen (microaearophilic). In addition, recent research has implicated *P. gingivalis* as the keystone pathogen of periodontitis, which is an inflammatory disease constituting complex dysbiotic microbial community residing below the gumline, within "pockets". *P. gingivalis* appears to translocate from the saliva to the subgingival location using neutrophils as "Trojan horses" in some individuals because clinical observations suggest that not everyone progresses to manifesting periodontal disease.

The mouth harbours a microbiome, which essentially is a reservoir of health promoting microbes until their balance changes to more pathogenic forms. The fact that *P. gingivalis* can act as a commensal,~~and~~ provides us with an opportunity to discuss ~~the role of its source of *Porphyromonas gingivalis*~~ its primary oral source to its access of the brain in relation to cognitive dysfunction. ~~This is not only because~~ AD is a prime example of a dementing neurological disease ~~but also for~~that has a plausible ~~the established~~ association with~~of~~ *P. gingivalis* ~~with both the AD brain~~ [11, 12]. ~~and periodontitis as a keystone bacterium [12]. In addition,~~ This is strengthened by the development of models for periodontal infection and AD in mice ~~*P. gingivalis* infection to the brain directly from its primary oral niche~~ [13] where it has been demonstrated to ~~can~~ reproduce the cardinal hallmark pathology inclusive of Aβ plaques, phosphotau [14]~~,~~ and cognitive function ~~in experimental mice~~ [15-17].

Alzheimer's disease

AD is end of life stage and the most common example of dementia. The cardinal clinical signs are cognitive decline with deterioration in memory. The h~~H~~ippocampus is the region of the brain where memory is processed and the functional loss has been associated with the death of neurons in specific regions of the brain related to memory. AD has a long preclinical phase (20 years) with the duration of suffering lasting on average for 8-10 years and longer [18]. At the preclinical stage of the illness, the individual may not seek medical help. Usually a family member or the caregiver~~r~~ of the person with declining cognition and memory may voice their concern to a health care professional. This may be their general medical practitioner (GP) or a health care professional (district nurse). The first stage in exploring this health complaint is for the caregiver~~r~~ to take the person (with suspected dementia signs) to his/her GP. The GP will then refer the person on to a memory service to establish a more formal clinical diagnosis, and initiate treatment and support. The final diagnosis of AD rests with both the clinical history together with the demonstration of the neuropathological occurrence of Aβ plaques and hyperphosphorylated tau protein binding to neurofibrillary tangles in a characteristic pattern and distribution in the specific regions of the brain. AD neuropathology can co-exist with other neurological and/or vascular pathologies because it is not an isolated disease.

Plausible cause of Alzheimer's disease and Lewy-body dementia

The cause of the sporadic forms of the neurological diseases under discussion (AD and Parkinson's disease with Lewy bodies) remains unclear. However, amongst others, the risk factors include ageing and inheritance of the apolipoprotein E gene allele 4 (*APOE ϵ4*) [19, 20]. The *APOE ϵ4* susceptibility gene links with environmental risk factors that include the host's dysbiotic oral microbiome [21]. *P. gingivalis* infections of the brain in laboratory mice induced with periodontitis demonstrate excessive oxidative stress and inflammation [13-15, 22].

Lewy bodies are intra-neuronal cytoplasmic inclusions composed of synuclein and other proteins lying within the pigmented neurons of the substantia nigra, limbic and the cerebral cortex regions of the brain. The clinical symptoms of Parkinson's disease in its purest form are tremor, immobility and rigidity of muscles. However, cognitive deficit occurs when Parkinson's disease co-exists with dementia (Lewy body Parkinsonian dementia), see comment above related to mixed pathologies. Epidemiological investigations [4, 5] in a

Taiwanese population have linked this to periodontal disease. As mentioned earlier, the Aβ protein of AD plaques and the α-synuclein within Lewy bodies are a form of broad-spectrum antimicrobial peptides, released following infection, including that caused by the periodontal pathogen *P. gingivalis* [7-10, 14]. If Aβ and α-synuclein represent the host's response to a previous infection, it follows that these neurodegenerative diseases have causative associations with microbes during their development. This has given rise to the antimicrobial protection hypothesis [23] linking infection as a plausible trigger for the sporadic form of AD. If this theory becomes widely accepted, then explaining the existing oxidative stress, the activated complement, the longstanding inflammation and the defects in the blood-brain barrier (BBB) would be easy in the context of *P. gingivalis* infection [13, 22, 24]. All of the above-mentioned signaling cascades and others (not included here), would enhance the role of Aβ as an antimicrobial peptide in killing the elusive invader(s) and/or the little understood brain's own microbiome converting to a pathobiome. In addition, the elderly are unlikely to be immuno-privileged because the BBB defects in the 70+ year's age group are associated with more rapid cognitive decline [25] and could have implications for pathogen entry.

Plausible cause of cognitive deficit

What actually causes the cognitive deficit during dementia onset is unclear, because the individual examples of dementia such as AD are seldom pure. However, the amyloid cascade hypothesis originally focused on Aβ deposits as a possible cause [26]. Subsequent immunological therapy to remove Aβ plaques from the brains of AD patients disproved the notion that insoluble Aβ deposits contribute to cognitive dysfunction [27]. Prior to the amyloid hypothesis, the synaptic loss hypothesis of Terry et al. [28] and Masliah et al. [29] originated from the fact that specific neuronal loss may be due to synaptic loss. The revised version of the amyloid cascade hypothesis has incorporated soluble oligomeric Aβ in the synaptotoxicity and cognitive impairment theory [30]. It is possible that there is close interplay between the mechanisms underlying these three hypotheses. After all, it is highly plausible that microbial debris, inflammatory mediators, oligomeric Aβ, smaller tau peptides released by gingipains, and pathogen activated inflammasomes [31], can all act to disrupt synapses and result in cognitive deficit.

Relationship between periodontitis and AD

The idea of dementia being a risk factor for periodontitis is undisputable, but then one would expect all demented individuals to have periodontitis by the time of death. Literature suggests the formerly known "chronic" periodontitis has a clearer relationship with a subgroup of AD cases [32-36]. Significant progress will only be made to find the actual direction of this relationship, once we better understand the parameters that should be included and/or excluded from the investigation in case control and/or cohort studies. For example, we now understand that periodontitis only becomes a risk factor for AD development some 10 years after it is diagnosed [37, 38]. This would imply that studies conducted in less than 10-year cohort analysis would provide inconclusive results [39]. One suggested risk of developing AD is having fewer remaining teeth (loss of up to 9 teeth) in early to mid-life due to periodontitis [30, 40], resulting from longstanding poor oral hygiene. For a more comprehensive discussion on the direction of the relationship between oral health and risk of developing AD, see Daly et al. [41]. There is agreement that periodontitis doubles the risk for developing late onset AD with an odds ratio of 2.2 (95% CI 1.1, 4.5) 10 years after its initial diagnosis [37, 38]. An interventional study on the periodontal treatment in AD patients [42] indicated a plausible causal relationship in demented individuals. It is suggested that patients with early stage dementia (at the time of~~point when they~~ visiting the memory clinic for initial diagnosis) show worsening oral hygiene [43], implying that dementia may be the risk factor for periodontal disease in this group of patients. It is also suggested that if dental intervention is provided at the early stage of dementia onset, it would delay the speed of cognitive deterioration. Early intervention is important and memory clinics should consider taking it on at the time the initial diagnosis [43]. However, to confirm the direction of the relationship, more studies with larger cohorts are needed in the "at risk" subpopulation of individuals whose periodontitis co-exists with AD cases. In addition, future interventional studies should include participants who suffer from periodontitis approaching the risk age for dementia (pre 65-year age) for maximal impact on delaying the onset of AD.

Relationship of *P. gingivalis* with AD development

As mentioned, *P. gingivalis* is considered a keystone pathogen in periodontitis [12] and it is adept at manipulating the sub-gingival microbiome and the host's immune system [44-49]. *P. gingivalis* is an intracellular pathogen that has been used to develop AD via periodontal infection in mice [13, 14]. The infection periodontal model of Ilievski et al. [14] produced the AD defining hallmark lesions in the mouse brains (Aβ and phosphotau neurofibrillary

tangles), a finding reproduced in mice by Dominy et al. [50]. Since the Ilievski and the Dominy models were of wild type mice, there is a high probability that Aβ was cleaved from its precursor protein into various oligomer sizes following oxidative stress initiated by *P. gingivalis*, which in turn activated cathepsin B within the endo/lysosomes [22, 51]. This intracellular processing of Aβ agrees with the earlier report of Wu et al. [15] showing, that metabolic processing of the amyloid precursor protein after *P. gingivalis* lipopolysaccharide (LPS) was administered into cathepsin B sufficient mice. Other studies in which either *P. gingivalis* or its LPS was introduced, supported the development of the AD-like clinical phenotype [15-17, 52] resulting in impaired spatial learning and memory. All of these investigations support a causal relationship of periodontitis with the development of AD.

Mechanisms of cognitive deficit by *P. gingivalis* infection

Soluble oligomeric Aβ and BBB defects

In line with Dominy et al. [50] confirming *P. gingivalis* genetic footprints (DNA) in the AD brains, *in vivo* infection models of periodontitis are recapitulating hallmark proteins and the emerging phenotype is supporting cognitive deficit [14-17, 52]. *P. gingivalis* produces two types of cysteine proteases (gingipains). They are the lysine specific Kgp and the arginine specific RgpA and RgpB gingipains [53]. A novel finding described by Dominy et al. [50] is the capacity of these proteases to hydrolyse the biochemical structure of the protein tau, and this opens up future avenues for research.

Gingipains activity has the potential to erode endothelial tight junction proteins [24] as supported by the *P. gingivalis*/host interactome study [54]. Cognitive deterioration due to BBB defects in the human elderly individuals are also documented [25] and this may yet be another contributory factor in mice models displaying AD-like clinical phenotype. In addition, if the soluble form of the olgomeric Aβ can interfere with synapses and contribute to cognitive deficit, as proposed by Cline et al. [30]. Then *P. gingivalis* oral infection can also contribute to this protein following its entry into the brain [14, 50].

Inflammation and inflammatory mediators in general

Numerous studies have shown that LPS from Gram negative bacteria either administered directly into the peritoneum or the brain, induce neuroinflammation in the form of glial cell activation [55] and when measured, the inflammatory response is accompanied by learning and memory impairment [56, 57] as a result of IL-1β secretion following peripheral challenge with LPS [58]. This is in agreement with the Wu et al. [15] hypothesis that systemic

administration of *P. gingivalis* LPS leads to cognitive deficit following Aβ liberation in an IL-1β receptor dependent pathway on neurons, (also see [21]). IL-1β cytokine is implicated in synaptic loss [59, 60] and with reduced long-term potentiation, which is a unit of memory [59], supporting the role of this cytokine in deteriorating cognition.

***P. gingivalis*, complement, and immune dysbiosis**

Gingipains are virulence factors of great importance to the immune subversion activity of *P. gingivalis* [53]. In the context of the complement cascade, these proteases play a major role. *P. gingivalis* oral infection of apolipoprotein $E^{-/-}$ mice demonstrated complement activation in their brains [13]. Activation of complement does take place in AD brains, where Aβ plaques are the suggested trigger [61]. If, according to the novel hypothesis of Allen [62] that Aβ senile plaques are miniature foci of bacterial biofilms, and that the antimicrobial protection theory of Moir et al. [23] supporting the Aβ antimicrobial peptide idea then the downstream immune activity triggering complement activation in AD brains does fit. Inappropriately activated complement compromises the function of healthy neurons, because of their inadequate shielding from protective proteins that rescue them from the non-specific mode of activity of this powerful innate immune signaling cascade [63]. During complement activation~~,~~ release of several small proteins (opsonins) takes place, which then opsonize to neurons [13]. Depending on the site of opsonin binding to the neuron, (e.g. at the synaptic cleft), there remains a potential to disrupt the path of neuronal communication and give way to cognitive dysfunction. In addition, the continuation of this cyclic cascade will generate more cytokines and contribute to cognitive deficit (see above).

P. gingivalis infection continues to cleave complement components (C1-C5) through its gingipains activity, and prevents both deposition of C3b on the bacterial surface and capture of the C4b binding protein [64-68]. By hijacking the complement regulator C4bp on the bacterial surface, *P. gingivalis* prevents assembly of the membrane attack complex and acquires the ability to regulate C3 convertase [66]. Accordingly, the gingipains do not only destroy complement through proteolytic degradation, but they also inhibit activation of complement by binding to the complement inhibitor C4bp [66]. This inhibits complement action and results in a local accumulation of the anaphylatoxin C5a [69]. *P. gingivalis* also exerts C5 convertase-like enzymatic activity and exploits complement-Toll like receptor (TLR) crosstalk to subvert host defenses and thus escape elimination from the host [45]. Zhang et al. [52] recently demonstrated that the mechanism by which *P. gingivalis* impaired

spatial learning and memory is via TLR crosstalk because inhibiting this pathway rescued memory in their infection mouse model.

As an analogy to TLR signaling, our in house data clearly showed that CD14, an LPS binding receptor, expressed on healthy IMR32 neurons (also participates in TLR signaling) was completely or partially removed following exposure to endo/exotoxins from *P. gingivalis* ATCC 33277^{T} and W50, respectively (see Figure 1). Such mechanisms lead to defective immune surveillance because of their influence in remodeling the periodontal microbiota into a dysbiotic state. *P. gingivalis* can also reduce the antibacterial and proinflammatory activity of C5a by deiminating its C-terminal arginine residues [70]. Degradation of complement proteins probably allows colonization and proliferation of bacteria possessing higher sensitivity towards complement killing than found in *P. gingivalis* itself [47]. Thus, *P. gingivalis* may support survival of the entire biofilm community by helping bystander bacteria evade complement mediated killing [46], whilst neurons survive with compromised function. These activities have consequences for the developing neuropathology. Thus, the neuropathology and the clinical functional loss together, constitute the AD diagnosis. *P. gingivalis* infection under laboratory conditions are supporting both of these possibilities [13-17, 22, 24, 52].

Bacterial factors disrupting synapses

Our in-house *in vitro* studies in which IMR32 (neuroblastoma-derived) neurons challenged with *P. gingivalis* virulence factors (containing LPS and gingipains) indicated considerable alterations in their actin cytoskeletal filaments following their detection with fluorescein-phalloidin dye. The LPS binding to cell surface membranes caused blebbing [11], whilst the protease caused the cells to withdraw their processes and round up (see Figure 2). In summary, the structural alteration of the IMR32 neurons, *in vitro*, could provide the basis for the failure of communication between neighboring cells. ~~In addition, excess bacterial/inflammatory mediators possibly trap between micro spaces of opposing (pre-post) synapses (synaptic clefts) or adversely affect synaptosomes during their neurotransmitter release contributing to cognitive loss. These areas are open to future investigations in relation to memory.~~ Infection of microglia with *P. gingivalis* in mice has promoted cell migration and an inflammatory response through gingipain-mediated activation of protease-activated receptor-2 [71]. We need to clarify if and how infectious episodes impair memory at the synaptosomal level, rather than at the synaptic cleft level. Such information may refine our

understanding at an earlier stage of deteriorating cognition albeit at the neurotransmitter release and its uptake levels.

Dysbiosis of immune defense by alternative means

miRNA has a role in the virulence of *P. gingivalis*, contributing to modulation of host-cell immune responses in a manner that promotes bacterial survival, and progressively reduces the host's protective function [49]. Some miRNAs are even associated with *P. gingivalis* itself [72], while others (miRNA-128, miRNA-146, miRNA-203, and miRNA-584) are host derived for inflammation. Bacterium-associated miRNAs are likely to influence the innate immune response against *P. gingivalis*, whereas LPS from this bacterium may affect the level of the host's miRNA–mRNA interactions. These miRNA-dependent effects may supplement other forms of deception exerted by *P. gingivalis* thus subverting innate and adaptive immune responses possibly by altering gene function [54, 69].

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***P. gingivalis* and tau protein phosphorylation**

As mentioned earlier, Ilievski et al. [14] demonstrated that *P. gingivalis* infection can lead to tau phosphorylation and neurofibrillary tangle formation in mice. The neurons that develop these hallmark lesions in the human AD brain are cells with compromised function, and the structural change in the nerve cell soma and axons, the later disrupting their connectivity. The effect of gingipains on the integrity of actin filaments seen with IMR32 neurons (Figure 2) may be analogous to the neurofibrillary tangle bearing neurons in AD. This structural change is likely to be detrimental to their communications with other brain cells resulting in deteriorated cognition.

Previously, we have discussed outer membrane vesicles (microbullets) from *P. gingivalis* [73] playing a role in AD development. *P. gingivalis* cultures produce them in vast numbers, suggesting they constitute the main superhighway of communication with other bacteria in the biofilm [74]. Since they carry additional arsenals of weapons to manipulate their entry into disparate organs, disrupt actin structures, erode epithelial junctional proteins, hijack phagocytosis, destroy tissues, and affect complement related genes, they may also be responsible for transducing proinflammatory signaling cascades that ultimately lead to disease defining lesion development and cognitive decline, typical of clinical AD.

Ilievski et al. [14] demonstrated a chronic infection with live *P. gingivalis* strain W83 for 22 weeks with both the hallmark lesions (Aβ and NFTs) that characterize AD with tau

protein phosphorylation at the serine396 (ser396) residue. This generated a new concept that an oral infective focus in neurological diseases may result in dementia. Up until now, abnormally phosphorylated tau protein has not featured negatively in the pathophysiology of periodontal disease *per se*. However, Adamowicz et al. [75] implicated the role of glycogen synthase kinase 3 (GSK-3) in bacterial-induced periodontitis because its inhibition rescued bone loss. Thus, GSK-3 may be influencing phosphorylation of brain tau via immune responses mediated by *P. gingivalis*, in the Ilievski et al. [14] study. GSK-3β appears to mediate proinflammatory cytokine production during bacterial infections because inhibition of GSK-3β leads to an innate hypo-reactivity to oral pathogens [76]. Macrophages treated with LPS, *in vitro* suggest that GSK-3β stimulates interferon-β (IFN-β) production via c-Jun thus activating a transcription factor (ATF)-2-dependent mechanism [76]. GSK-3β also negatively regulates production of the endogenous IL-1β antagonist, IL-1R, via its ability to regulate the MAPK and ERK 1/2 in LPS-stimulated innate immune cells. There is no doubt that further research will widen investigation of these pathways for more direct causal links with oral disease and dementing diseases with cognitive deterioration.

The Dominy et al. [50] publication has provided a stronger argument for the role of pathogenic tau in AD development. In their *in vitro* neuronal culture system, Dominy et al. [50] demonstrate that tau is a substrate for gingipains and show a low molecular weight band corresponding to a novel tau peptide. Further research will establish if it is neurotoxic or not.

P. gingivalis **and lymphocytes**

It is possible that T cell entry into the AD brain is restricted and this somehow influences ineffective clearance of the Aβ by macrophages and the resident microglia. Baek et al. [77] found that Treg cells (subpopulation of T cells) had an effect on cognitive function by decreasing Aβ deposition and inflammatory cytokine secretion in a 3xTg-AD mice model. In contrast, depletion of Tregs increased the onset of cognitive deficit, accelerated the amount of the Aβ burden, enhanced microglia/macrophage responses and decreased glucose metabolism in 3xTg-AD mice. In patients with atherosclerosis, the Treg population was reduced if they harbored type II fimA of *P. gingivalis* compared to those with other types of fimbriae [78]. Therefore, *P. gingivalis* type II fimA could be associated with dysregulation of Tregs in extraoral lesions. Severe immunosuppression seems to favor not only colonization with varying serotypes of periodontopathogenic bacteria, but also with species not commonly found in the subgingival microbiota [79]. In the brain, this may contribute to the

establishment of a multi species microbiota, previously reported in AD patients [80]. In addition, accumulation of insoluble and toxic Aβ42 has detrimental effect on the neighboring neurons and their connections, which may have further implications for neurodegeneration and related cognitive loss.

Conclusions

Dominy et al. [50] have recently provided robust data linking the main pathogen (*P. gingivalis*) of periodontitis with the cause of AD. This bacterium appears to migrate from the mouth to the brain of some individuals as they age and a significant proportion of subjects who go onto developing AD. This further highlights the possibility that AD has a microbial infection origin. Ilievski et al. [14] provide evidence for *P. gingivalis* infection having causal associations by reproducing the hallmark lesions. Four independent studies carried out in mice infected with *P. gingivalis* provide causal links through impaired learning and memory. The suggested mechanism is related to the TLR crosstalk and this may have relevance to the inflammasome formation with the resulting cytokines (mature IL-1β) being linked to memory disturbances.

These studies reinforce the advice that oral hygiene is important in keeping pathogens low and encouraging greater diversity of commensals (health promoting bacteria). This provides a healthy microbiome and better general health. Health authorities need to heed this warning and take research based evidence seriously. The UK NHS England provides a recommendable oral health toolkit for the elderly to maintain better oral hygiene with the aim of delaying/preventing AD.

Conflict of Interest The authors declare no conflict of interest.

Human and Animal Rights and Informed Consent

Not applicable. This is a review of literature and does not rely on freshly obtained data from human and/or animal studies. Figures are from our in-house cell culture studies that are exempt from ethical issues.

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Figure legends

Figure 1. Western blot showing CD14 protein on the human neuroblastoma cell line IMR32. **a**) is an immunoblot of cell lysate prepared from IMR32 neurons following their standard growth culture medium and incubation conditions, ~~no exposure to virulence factors (control)~~ (lane 1). ~~and c~~IMR32 neurons cultured in their growth medium to which ~~ontrol with~~ *P. gingivalis* sterile growth medium diluted 1:4 from stock~~for *P. gingivalis*~~ was added~~cultures~~ (lane 2).~~,~~ IMR32 neurons in their growth medium plus *P. gingivalis* ATCC 33277^{T} conditioned medium diluted 1:4 from stock (lane 3) ~~with exposure (test) to *P. gingivalis* ATCC 33277^{T} (lane 3)~~ and strain W50 conditioned medium (diluted 1:4 from stock) (lane 4) ~~spent medium (diluted 1:4 from stock)~~ for 24 h. The proteins were separated by SDS-PAGE electrophoresis and electro transferred onto the PVDF (polyvinylidene difluoride) membrane. Following incubation of the membrane overnight with mouse anti-CD14 antibody, clear bands around the 55 kDa molecular weight were seen (in the control lanes 1 and 2, long arrow) indicates~~ing~~ that the CD14 receptor protein was expressed ~~present on control~~ by these cells. Upon challenge with *P. gingivalis* 33277^{T} the band completely diminished (lane 3, CD14 cleaved from cell membrane). Treatment of the same cells with the W50 strain surprisingly, only partially cleaved CD14 (lane 4) as compared with the control lanes 1 and 2. **b**) IMR32 cells grown on coverslips were also incubated with the same anti-CD14 antibody. The green colour shows CD14 labelling on the surface membrane of cells confirming~~meaning~~ that the receptor is intact. The

red colour indicates the nucleus due to propidium iodide uptake from the mounting medium. **c**) Following exposure to *P. gingivalis* 33277^{T}, the cells for 24 h (as for the blot), the green labelling was missing and correlated with the blot data. **d**) Exposure to *P. gingivalis* W50, demonstrated green labelling on the membranes again correlating with the blot data.

Figure 1.

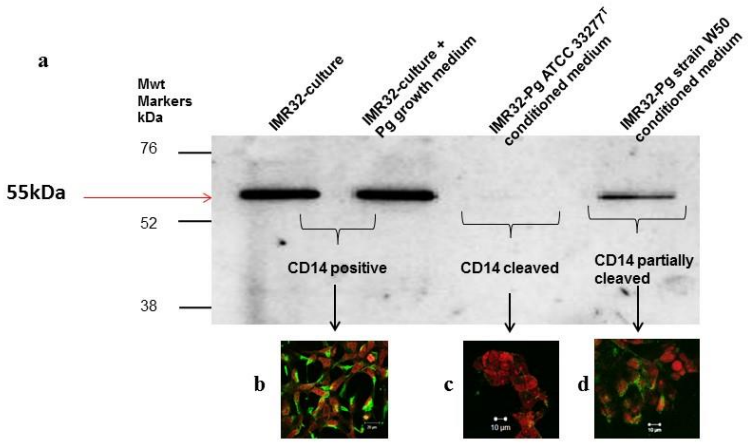
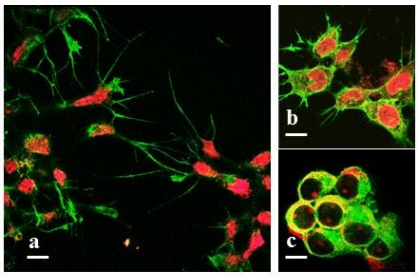


Figure 2

IMR32 neurons in culture: Fluorescein-phalloidin (5 units/ml final, for 30 min) labelling for actin cytoskeletal protein (green), (nuclei = red due to propidium iodide uptake). **a**) IMR32 monolayer in growth medium shows long processes of the cells extending outwards. **b**) Exposure to *P. gingivalis* ATCC 33277^{T}, spent medium (diluted 1:4) for 6 h demonstrated the processes thickened, whilst the cell soma enlarged. **c**) As for b, but after 24 h exposure, the cells rounded up and detached. Images taken after examining the cells under the 510 series Zeiss

754 confocal microscope (Carl Zeiss Ltd). Micron bar = 10

Figure 2.



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